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Ambient Water Quality Criteria for Nitrobenzene

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16. ABSTRACT

Section 304(A) of the Clean Water Act [33 U.S.C. 1314(A)], requires EPA to publish and periodically update water quality criteria. These criteria are to reflect the latest scientific knowledge on the identifiable effects of pollutants on public health and welfare, aquatic life, and recreation. This report presents water quality criteria for Nitrobenzene. It presents concentration criteria for the protection of freshwater and saltwater aquatic life. It presents 'safe' concentrations for humans, and in the case of suspect or proven carcinogens, gives various levels of incremental cancer risk. A Section 304(A) water quality criterion is a qualitative or quantitative estimate of the concentration of a water constituent or pollutant in ambient waters which, when not exceeded, will ensure a water quality sufficient to protect a specified water use. Under the Act a criterion is a scientific entity, based solely on data and scientific judgment. It does not reflect considerations of economic or technological feasibility nor is it a water quality standard and in itself has no regulatory effect.

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AMBIENT WATER QUALITY CRITERIA FOR

NITROBENZENE

Prepared By
U.S. ENVIRONMENTAL PROTECTION AGENCY

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FOREWORD

Section 304 (a)(1) of the Clean Water Act of 1977 (P.L. 95-217), requires the Administrator of the Environmental Protection Agency to publish criteria for water quality accurately reflecting the latest scientific knowledge on the kind and extent of all identifiable effects on health and welfare which may be expected from the presence of pollutants in any body of water, including ground water. Proposed water quality criteria for the 65 toxic pollutants listed under section 307 (a)(1) of the Clean Water Act were developed and a notice of their availability was published for public comment on March 15, 1979 (44 FR 15926), July 25, 1979 (44 FR 43660), and October 1, 1979 (44 FR 56628). This document is a revision of those proposed criteria based upon a consideration of comments received from other Federal Agencies, State agencies, special interest groups, and individual scientists. The criteria contained in this document replace any previously published EPA criteria for the 65 pollutants. This criterion document is also published in satisifaction of paragraph 11 of the Settlement Agreement in Natural Resources Defense Council, et. al. vs. Train, 8 ERC 2120 (D.D.C. 1976), modified, 12 ERC 1833 (D.D.C. 1979).

The term "water quality criteria" is used in two sections of the Clean Water Act, section 304 (a)(1) and section 303 (c)(2). The term has a different program impact in each section. In section 304, the term represents a non-regulatory, scientific assessment of ecological effects. The criteria presented in this publication are such scientific assessments. Such water quality criteria associated with specific stream uses when adopted as State water quality standards under section 303 become enforceable maximum acceptable levels of a pollutant in ambient waters. The water quality criteria adopted in the State water quality standards could have the same numerical limits as the criteria developed under section 304. However, in many situations States may want to adjust water quality criteria developed under section 304 to reflect local environmental conditions and human exposure patterns before incorporation into water quality standards. It is not until their adoption as part of the State water quality standards that the criteria become regulatory.

Guidelines to assist the States in the modification of criteria presented in this document, in the development of water quality standards, and in other water-related programs of this Agency, are being developed by EPA.

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CRITERIA DOCUMENT

NITROBENZENE

CRITERIA

Aquatic Life

The available data for nitrobenzene indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as 27,000 µg/l and would occur at lower concentrations among species that are more sensitive than those tested. No definitive data are available concerning the chronic toxicity of nitrobenzene to sensitive freshwater aquatic life.

The available data for nitrobenzene indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 6,680 µg/l and would occur at lower concentrations among species that are more sensitive than those tested. No definitive data are available concerning the chronic toxicity of nitrobenzene to sensitive saltwater aquatic life.

Human Health

For comparison purposes, two approaches were used to derive criterion levels for nitrobenzene. Based on available toxicity data, for the protection of public health, the derived level is 19.8 mg/l. Using available organoleptic data, for controlling undesirable taste and odor qualities of ambient water, the estimated level is 30 µg/l. It should be recognized that organoleptic data as a basis for establishing a water quality criterion have limitations and have no demonstrated relationship to potential adverse human health effects.

INTRODUCTION

Nitrobenzene is produced for industrial use by the nitration of benzene with nitric and sulfuric acids. Estimates of annual nitrobenzene production range from 200 to over 700 million pounds (Dorigan and Hushon, 1976; Lu and Metcalf, 1975). The principal use of nitrobenzene is for reduction to aniline, which is widely used as an ingredient for dyes, rubber, and medicinals (McGraw-Hill, 1971; Kirk and Othmer, 1967). The commercial applications of nitrobenzene are: reduction to aniline (97 percent), solvent for Friedel-Crafts reaction, metal polishes, shoe black, perfume, dye intermediates, crystallizing solvent for some substances, and as a combustible propellant (Dorigan and Hushon, 1976).

Nitrobenzene is stored in closed containers and is not usually released to the open air. Atmospheric contamination is usually prevented in plants manufacturing or using nitrobenzene by the use of activated charcoal absorbers or a carbon dioxide blanket. There is no industrial monitoring of nitrobenzene in the atmosphere. The greatest loss of nitrobenzene during production (estimated as eight million pounds annually) occurs at the acid extraction step in the purification of the crude reaction mixture, when nitrobenzene is lost to the effluent wash (Dorigan and Hushon, 1976). Thus, the greatest exposure to nitrobenzene occurs inside plants and most cases of chronic nitrobenzene exposure in man are nitrobenzene workers. Today plant levels of nitrobenzene are usually kept below the threshold limit value (TLV) of 5 mg/m^3 [Goldstein, 1975; American Conference of Governmental Industrial Hygienists (ACGIH), 1977] but much higher levels have been reported in the past (Pacseri and Magos, 1958). Nitrobenzene may also form spontaneously in the atmosphere from the photochemical reaction of benzene with oxides of nitrogen.

Nitrobenzene, also known as nitrobenzol, essence of mirbane, and oil of mirbane, is a pale yellow oily liquid with an almond-like odor (Kirk and Othmer, 1967). The color of the liquid varies from pale yellow to yellowish brown depending on the purity of the compounds (Kirk and Othmer, 1967). In the solid state it forms bright yellow crystals. Nitrobenzene, $C_6H_5NO_2$, has a molecular weight of 123.11 g.

The physical properties of nitrobenzene are as follows: a boiling point of 210° to 211°C at 760 mm Hg, a melting point of 6°C, a density of 1.205 at 15°C, a refractive index of 1.5529, and a flash point of 89°C (Stecher, 1968). It is steam volatile (Stecher, 1968) and at 25°C nitrobenzene has a vapor pressure of 0.340 mm Hg (Jordan, 1954).

Nitrobenzene is miscible with most organic solvents, such as ethanol, diethyl ether, acetone, and benzene (Kirk and Othmer, 1967). It is slightly soluble in water, 0.1 per 100 parts of water (1,000 mg/l) at 20°C (Kirk and Othmer, 1967). In aqueous solutions, nitrobenzene has a sweet taste (Kirk and Othmer, 1967).

Nitrobenzene undergoes substitution reactions but requires more vigorous conditions than does benzene. Substitution takes place at either the meta-(3) position or the ortho-(2) or para-(4) positions depending on the physical conditions (Kirk and Othmer, 1967). Nitrobenzene undergoes photo-reduction when irradiated with ultraviolet light in organic solvents that contain abstractable hydrogen atoms (Barltrop and Bunce, 1968).

Nitrobenzene is a fairly strong oxidizing agent (Kirk and Othmer, 1967; Millar and Springfield, 1966). Since the compound can act as an oxidizing agent in the presence of aqueous solutions of alkali hydroxides, it has the capability of oxidizing compounds containing free phenolic hydroxyl groups without effectively changing these groups (Millar and Springfield, 1966).

Nitrobenzene is rective and will undergo nitration, halogenation, and sulfonation by the same methods used for benzene. However, these reactions are unlikely to occur in environmental conditions.

The reduction of nitrobenzene to aniline probably outranks all other uses of nitrobenzene as an industrial chemical (Kirk and Othmer, 1967). The di- and the trinitrobenzenes are used in military and industrial explosives.

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Aquatic Life Toxicology*

INTRODUCTION

Static tests with the bluegill, Daphnia magna, and the alga, Selenastrum capricornutum, indicate little difference in sensitivity with no 50 percent effect concentration lower than 27,000 µg/l. An embryo-larval test with the fathead minnow demonstrated no adverse effects at the highest test concentration of 32,000 µg/l.

Static acute tests with the sheepshead minnow and Mysidopsis bahia indicate that the latter is much more sensitive to nitrobenzene. Adverse effects were observed on a saltwater alga at concentrations slightly higher than the LC_{50} for the mysid shrimp.

EFFECTS

Acute Toxicity

The 48-hour EC_{50} for Daphnia magna and the 96-hour LC_{50} for the bluegill are 27,000 and 42,600 µg/l, respectively (Table 1).

The saltwater species are comparable to the freshwater species in their sensitivity to nitrobenzene. The mysid shrimp LC_{50} is 6,680 µg/l (Table 1) and the LC_{50} for the sheepshead minnow is 58,600 µg/l.

Chronic Toxicity

No adverse effects were observed during an embryo-larval test with the fathead minnow at test concentrations of nitrobenzene as high as 32,000 µg/l (Table 2).

*The reader is referred to the Guidelines for Deriving Water Quality Criteria for the Protection of Aquatic Life and Its Uses in order to better understand the following discussion and recommendation. The following tables contain the appropriate data that were found in the literature, and at the bottom of each table are calculations for deriving various measures of toxicity as described in the Guidelines.

Plant Effects

The 96-hour EC_{50} values for reduction of cell numbers and inhibition of chlorophyll *a* in the freshwater alga, *Selenastrum capricornutum*, are 42,800 and 44,100 µg/l, respectively (Table 3).

The cell numbers of *Skeletonema costatum* were reduced by 50 percent at a concentration of 9,650 µg/l (Table 3). Chlorophyll *a* was equally inhibited at a concentration of 10,300 µg/l.

Summary

The acute 50 percent effect levels of *Daphnia magna* and the bluegill were 27,000 and 42,600 µg/l, respectively. No effects on fathead minnow embryos or larvae were observed at concentrations as high as 32,000 µg/l. A freshwater alga was of similar sensitivity with an EC_{50} value for chlorophyll *a* of 44,100 µg/l.

Ninety-six-hour LC_{50} values were 6,680 and 58,600 µg/l for the mysid shrimp and sheepshead minnow, respectively. The EC_{50} for cell numbers of a saltwater alga was 9,650 µg/l.

CRITERIA

The available data for nitrobenzene indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as 27,000 µg/l and would occur at lower concentrations among species that are more sensitive than those tested. No definitive data are available concerning the chronic toxicity of nitrobenzene to sensitive freshwater aquatic life.

The available data for nitrobenzene indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 6,680 µg/l and would occur at lower concentrations among species that are more sensitive than those tested. No data are avialable concerning the chronic toxicity of nitrobenzene to sensitive saltwater aquatic life.

Table 1. Acute values for nitrobenzene (U.S. EPA, 1978)

Species	Method*	LC50/EC50 (µg/l)	Species Acute Value (µg/l)
	FRESHWATER SPECIES		
Cladoceran, *Daphnia magna*	S, U	27,000	27,000
Bluegill, *Lepomis macrochirus*	S, U	42,600	42,600
	SALTWATER SPECIES		
Mysid shrimp, *Mysidopsis bahia*	S, U	6,680	6,680
Sheepshead minnow, *Cyprinodon variegatus*	S, U	58,600	58,600

* S = static, U = unmeasured

No Final Acute Values are calculable since the minimum data base requirements are not met.

Table 2. Chronic values for nitrobenzene (U.S. EPA, 1978)

Species	Method*	Limits (µg/l)	Chronic Value (µg/l)
FRESHWATER SPECIES			
Fathead minnow, *Pimephales promelas*	E-L	>32,000	-

* E-L = embryo-larval

No acute-chronic ratio is calculable.

Table 3. Plant values for nitrobenzene (U.S. EPA, 1978)

Species	Effect	Result (µg/l)
FRESHWATER SPECIES		
Alga, *Selenastrum capricornutum*	96-hr EC50 chlorophyll *a*	44,100
Alga, *Selenastrum capricornutum*	96-hr EC50 cell numbers	42,800
SALTWATER SPECIES		
Alga, *Skeletonema costatum*	96-hr EC50 cell numbers	9,650
Alga, *Skeletonema costatum*	96-hr EC50 chlorophyll *a*	10,300

REFERENCES

U.S. EPA. 1978. In-depth studies on health and environmental impacts of selected water pollutants. U.S. Environ. Prot. Agency, Contract No. 68-01-4646.

Mammalian Toxicology and Human Health Effects

INTRODUCTION

Nitrobenzene, a pale yellow liquid at room temperature with a characteristic bitter almond aroma, is also known as oil of mirbane, nitrobenzol, and artificial bitter almond oil. It is produced for industrial use by the nitration of benzene with nitric and sulfuric acids. Estimates of annual nitrobenzene production range from 200 to over 700 million pounds (Dorigan and Hushon, 1976; Lu and Metcalf, 1975). The principal use of nitrobenzene is for reduction to aniline, which is widely used as an ingredient for dyes, rubber, and medicinals. The commercial applications of nitrobenzene are: reduction to aniline (97 percent), solvent for Friedel-Crafts reaction, metal polishes, shoe black, perfumes, dye intermediates, crystallizing solvent, and as a combustible propellant (Dorigan and Hushon, 1976).

Nitrobenzene is stored in closed containers and not usually released to the open air. In plants manufacturing or using nitrobenzene, atmospheric contamination is usually prevented by the use of activated charcoal absorbers or a carbon dioxide blanket. There is no industrial monitoring of nitrobenzene in the atmosphere. The greatest loss of nitrobenzene during production (estimated as eight million pounds annually) occurs at the acid extraction step in the purification of the crude reaction mixture, when nitrobenzene is lost to the effluent wash (Dorigan and Hushon, 1976). Thus, the greatest exposure to nitrobenzene occurs inside plants, while most cases of chronic nitrobenzene exposure in man involve nitrobenzene workers. Today, plant levels of nitrobenzene are usually kept below the threshold limit value (TLV) of 5 mg/m^3 [Goldstein, 1975; American Conference of Governmental Industrial Hygienists (ACGIH), 1977] but much higher levels have been reported in the past (Pacseri and Magos, 1958). Nitrobenzene may also form

spontaneously in the atmosphere from the photochemical reaction of benzene with oxides of nitrogen; the symptoms of nitrobenzene poisoning are similar to the symptoms experienced by victims of Japanese photochemical smog (Dorigan and Hushon, 1976).

Nitrobenzene can be detected for monitoring purposes by colorimetric reaction, or by collection on a charcoal filter, extraction, reduction to aniline, and production of a colored product by diazotization of the aniline. These methods can detect nitrobenzene from 1.0 to 500 mg/m^3 (0.2 to 100 ppm) (Dorigan and Hushon, 1976). Nitrobenzene in wastewater can be measured by gas chromatography (Austern, et al. 1975). Exposure of workers to nitrobenzene is monitored by urinary levels of p-nitrophenol (Piotrowski, 1967) and p-aminophenol (Pacseri and Magos, 1958).

Some of the physical and chemical properties of nitrobenzene are summarized in Table 1. Common derivatives of nitrobenzene (besides aniline) are dinitrobenzene, nitrobenzene-sulfonic acid, and nitrochlorobenzene. There are many other derivatives of nitrobenzene, and many of them are very hazardous to man as toxic agents, mutagens, and carcinogens.

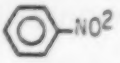
EXPOSURE

Ingestion from Water

Nitrobenzene can be released into wastewater from production plants as the result of losses during the production of nitrobenzene, aniline, or dyestuffs. The solubility of nitrobenzene is low, and it produces a detectable odor in water at a concentration as low as 0.03 mg/l (Austern, et al. 1975; U.S. EPA, 1970; Alekseeva, 1964), so that large amounts can not readily accumulate unnoticed. Levels of nitrobenzene in wastewater are monitored by plants producing and using the chemical but nitrobenzene levels in city

TABLE 1

Properties of Nitrobenzene

Formula:	$C_6H_5NO_2$ or 
Molecular weight:	123.11
Freezing point:	5.6 - 5.7°C
Boiling point:	210.9°C at 760 torr
Water solubility:	0.1 - 0.2 gm/100 ml at 20°C 1.0 gm/100 ml at 100°C
Soluble in:	ethanol, diethyl ether, acetone, benzene, lipids
Vapor pressure:	0.284 mmHg at 25°C 600 mmHg at 200°C
Vapor density:	4.24 (air = 1.0)
Log partition co-efficient:	hexane/water - 3.18 at 24.4°C
Density:	1.199 gm/ml at 25°C
Flash point:	87.8°C
Autoignition temp:	482.2°C
Viscosity:	1.682 cp at 30°C
Detection level of characteristic bitter almond odor:	10^{-4} mmoles/l

*Source: Dorigan and Hushon, 1976

water systems are usually too low to measure (Pierce, 1979). Nitrobenzene in water from an industrial spill is removed by treatment with activated charcoal.

There are no data available on mammalian toxicity of nitrobenzene ingested in drinking water.

Ingestion from Food

There are reports of nitrobenzene poisoning resulting from its uses as false almond oil in baking, rubbing on the gums to ease toothache, contamination of alcoholic drinks, and contamination of food (Nabarro, 1948). Leader (1932) reported a case of nitrobenzene poisoning in a child who was given "oil of almonds" for relief of a cold. Acute nitrobenzene poisoning has also occurred from ingestion of denatured alcohol (Donovan, 1920; Wirtschafter and Wolpaw, 1944). These cases are typical of accidental nitrobenzene ingestion. Nitrobenzene is not an approved food additive (Dorigan and Hushon, 1976).

A bioconcentration factor (BCF) relates the concentration of a chemical in aquatic animals to the concentration in the water in which they live. The steady-state BCFs for a lipid-soluble compound in the tissues of various aquatic animals seem to be proportional to the percent lipid in the tissue. Thus, the per capita ingestion of a lipid-soluble chemical can be estimated from the per capita consumption of fish and shellfish, the weighted average percent lipids of consumed fish and shellfish, and a steady-state BCF for the chemical.

Data from a recent survey on fish and shellfish consumption in the United States were analyzed by SRI International (U.S. EPA, 1980). These data were used to estimate that the per capita consumption of freshwater and estuarine fish and shellfish in the United States is 6.5 g/day (Stephan,

1980). In addition, these data were used with data on the fat content of the edible portion of the same species to estimate that the weighted average percent lipids for consumed freshwater and estuarine fish and shellfish is 3.0 percent.

No measured steady-state bioconcentration factor (BCF) is available for nitrobenzene, but the equation "Log BCF = (0.85 Log P) - 0.70" can be used (Veith et al., 1979) to estimate the BCF for aquatic organisms that contain about 7.6 percent lipids (Veith, 1980) from the octanol/water partition coefficient (P). Based on an average measured log P value of 1.84 (Hansch and Leo, 1979; Dec, et al., Manuscript), the steady-state bioconcentration factor for nitrobenzene is estimated to be 7.31. An adjustment factor of 3.0/7.6 = 0.395 can be used to adjust the estimated BCF from the 7.6 percent lipids on which the equation is based to the 3.0 percent lipids that is the weighted average for consumed fish and shellfish. Thus, the weighted average bioconcentration factor for nitrobenzene and the edible portion of all aquatic organisms consumed by Americans is calculated to be 7.31 x 0.395 = 2.89.

Inhalation

Nitrobenzene is readily absorbed through the lungs with retention of up to 80 percent (Piotrowski, 1967). There are reports of nitrobenzene poisoning from inhalation of an exterminator spray for bedbugs which was sprayed on a child's mattress (Stevenson and Forbes, 1942; Nabarro, 1948). Poisonings have also resulted from inhaled nitrobenzene used as a scent in perfume and soap (Dorigan and Hushon, 1976). Chronic and acute poisonings from exposure to nitrobenzene vapor in production plants are well documented (Dorigan and Hushon, 1976; Browning, 1950; Zeligs, 1929; Hamilton, 1919), but since nitrobenzene is also absorbed through the skin, industrial poisoning cannot be attributed to inhalation alone. A worker exposed to nitrobenzene

at 5 mg/m^3, the current Occupational Safety and Health Administration (OSHA) standard (40 CFR 1910.1000), would absorb 18 mg/day through the lungs in 6 hours (Piotrowski, 1967).

Dermal

Nitrobenzene is highly fat-soluble and can be absorbed through the skin at rates as high as 2 $mg/cm^2/hr$ (Dorigan and Hushon, 1976). Medical literature contains many reports of poisonings from absorption of nitrobenzene in shoe dyes and laundry marking ink. These reports were common during the 19th century and the first half of this century.

There have been reports of cases of shoe dye poisoning in an army camp (Levin, 1927), and in children who were given freshly dyed shoes (Zeitoun, 1959; Graves, 1928; Levin, 1927). The most frequent signs and symptoms were dizziness, bluish color of lips and nails (cyanosis), headache, and sometimes coma.

Cyanosis and poisoning of newborns who came in contact with diapers or pads containing marking ink were very common. Generally this occurred when the diapers or pads were freshly stamped by the hospital laundry (Etteldorf, 1951; Ramsay and Harvey, 1959; MacMath and Apley, 1954; Zeligs, 1929; Rayner, 1886). Often the imprint of the ink could be seen on the infant's skin. Removal of the diaper or pad and thorough washing of the skin usually reduced toxic symptoms, although methylene blue and ascorbic acid have also been used to relieve cyanosis. The toxicity is often more severe in premature infants who are in an incubator and exposed to the vapor as well as to the dye on the cloth (Etteldorf, 1951). Washing of the marked diapers or pads before their use removes the hazard of absorption of nitrobenzene or aniline from the ink.

In Egypt, "pure bitter almond oil" (a mixture of 2 to 10 percent nitrobenzene and 90 to 98 percent cottonseed oil) has been rubbed on babies to remove crusts from the skin and to protect the children from other diseases. Zeitoun (1959) reported cases of nitrobenzene poisoning seen in Alexandria hospitals as a result of this practice.

Hamilton (1919) reported a case of chronic nitrobenzene poisoning in a woman who used it as a cleaning fluid for many years. The continuous dermal absorption caused her to experience symptoms of multiple neuritis, extreme indigestion and hemorrhages of the larynx and pharynx.

Dermal absorption of nitrobenzene is the cause of many of the chronic and acute toxic effects seen in nitrobenzene workers (inhalation also accounts for industrial toxicity although the routes of exposure often cannot be distinguished). The amount of cutaneous absorption is a function of the ambient concentration, the amount of clothing worn, and the relative humidity (high humidity increases absorption) (Dorigan and Hushon, 1976). A worker exposed to the current OSHA standard (40 CFR 1910.1000), 5 mg/m^3, could absorb up to 25 mg in six hours, and one-third of that amount would pass through the skin of a clothed man (Piotrowski, 1967). Pacseri and Magos (1958) measured ambient nitrobenzene in industrial plants and found levels of up to eight times the current limit.

Hamilton (1919) reported a case of acute, fatal, nitrobenzene poisoning that resulted from a soap factory worker spilling "oil of mirbane" on his clothes. Immediate removal of the contaminated clothing would probably have prevented his death.

There are reports of acute and chronic poisoning due to skin absorption of dinitrobenzene by workers in munitions and nitrobenzene plants. Dinitrobenzene is believed to be much more toxic than nitrobenzene (Malden, 1907).

Ishihara, et al. (1976) reported a case of poisoning where a worker handled a cleaning mixture containing 0.5 percent dinitrobenzene. The worker wore gloves, but the dinitrobenzene penetrated the gloves to cause acute symptoms of methemoglobinemia and hemolytic jaundice. Rejsek (1947) described dinitrobenzene diffusion through the skin of munitions workers. Some of these workers with chronic dinitrobenzene poisoning experienced an acute crisis after exposure to sun or drinking alcohol (beer). Alcohol ingestion or chronic alcoholism can also lower the lethal or toxic dose of nitrobenzene (Dorigan and Hushon, 1976). This acute reaction could occur as late as six weeks after toxic symptoms disappeared.

Although there are many literature references dealing with occupational exposure to nitrobenzene, there are few, if any, reports of nitrobenzene exposure resulting from water intake. Therefore, data derived from occupational exposure will be used to develop information for establishing the water quality criterion in this document.

PHARMACOKINETICS

Absorption

Nitrobenzene absorption can occur by all possible routes, but it takes place mainly through the respiratory tract and skin. At 5 mg/m^3, a nitrobenzene worker can absorb 18 mg through the lungs and 7 mg through the skin in 6 hours (Piotrowski, 1967). On the average, 80 percent of the nitrobenzene vapor is retained in the human respiratory tract (Piotrowski, 1977).

Nitrobenzene, as liquid and vapor, will pass directly through the skin. The rate of vapor absorption depends on the air concentration, ranging from 1 mg/hr at 5 mg/m^3 concentration to 9 mg/hr at 20 mg/m^3. Air temperature does not affect the absorption rate, but an increase of relative humidity from 33 to 67 percent will increase the absorption rate by 40

percent. Work clothes reduce cutaneous absorption of nitrobenzene vapors by 20 percent (Piotrowski, 1977).

Maximal cutaneous absorption of liquid nitrobenzene is 0.2 to 3 $mg/cm^2/hr$ depending on skin temperature. Elevated skin temperature will increase absorption. Absorption will decrease with duration of contact. Cutaneous absorption can be significant in industry, since contamination of the skin and clothing of dye manufacture workers may reach levels of 2 and 25 mg/cm^2, respectively (Piotrowski, 1977).

Distribution

Upon entry into the body, nitrobenzene enters the bloodstream, where it reacts with the hemoglobin to form its oxidation product, methemoglobin. Methemoglobin has a reduced affinity for oxygen, and the reduced oxygen carrying capacity of the blood is the cause of most of the toxic effects of nitrobenzene, including its lethality. Methemoglobin levels from nitrobenzene have ranged from 0.6 gm/100 ml in industrial chronic exposure to 10 gm/100 ml in acute poisoning (Pacseri and Magos, 1958; Myslak, et al. 1971). The normal methemoglobin level is 0.5 gm/100 ml. Under normal conditions methemoglobin will slowly be reduced to oxyhemoglobin, the normal form of blood hemoglobin.

Pacseri and Magos (1958) have demonstrated that sulfhemoglobin is also formed in the blood after chronic exposure to nitrobenzene. In nitrobenzene workers, they found average sulfhemoglobin levels of 0.27 gm/100 ml (compared to the upper limit of normal of 0.18 gm/100 ml). Pacseri postulated that since blood sulfhemoglobin disappears more slowly than methemoglobin, it is a more sensitive indicator of nitrobenzene exposure. Sulfhemoglobin may be more specific than sensitive because methemoglobin is normally found in the blood whereas sulfhemoglobin is not.

Uehleke (1964) measured the velocity of methemoglobin formation from nitrobenzene in cats. He found the rate to be variable and not related to the blood concentration of nitrobenzene, although the methemoglobin formation velocity was maximal in each animal at the time of highest blood concentration of nitrobenzene. He also found that metabolites of nitrobenzene are able to oxidize hemoglobin. Methemoglobin formation from nitrobenzene has also been demonstrated in vitro (Dorigan and Hushon, 1976, Kusumoto and Nakajima, 1970).

Further indications of the presence of nitrobenzene in the blood are the production of hemolytic anemia after acute exposure (Harrison, 1977) and the alteration of the sodium and potassium permeability of erythrocytes by derivatives of nitrobenzene (Cooke, et al. 1968).

Nitrobenzene is very lipid soluble, with an oil to water partition coefficient of 800. In a rat study, the ratio of the concentration of nitrobenzene in adipose tissue versus blood in internal organs and muscle was approximately 10:1 one hour after an intravenous administration (Piotrowski, 1977). Rabbits intubated with 0.25 ml of nitrobenzene had 50 percent of the compound accumulated unchanged in tissues within two days after the intubation (Dorigan and Hushon, 1976).

Dresbach and Chandler (1918) have shown cerebellar disturbances in dogs and birds exposed to nitrobenzene vapor. A histologic study attributed these effects to changes in the Purkinje cells of the cerebellum. Reports of the effect of nitrobenzene on the liver vary from description of liver damage from accumulated nitrobenzene (Dorigan and Hushon, 1976) to the statement that nitrobenzene does not cause severe renal or liver damage (Goldstein, 1975). Goldwater (1947) has described hyperplasia of the erythropoietic centers of the bone marrow in workers chronically exposed to ni-

trobenzene, but he concluded that the hyperplasia is a secondary result of the hemolytic effect of the compound. Makotchenko and Akhmetov (1972) observed secretory changes of the adrenal cortex of guinea pigs given nitrobenzene every other day at a dose of 0.2 gm/kg for six months.

Metabolism

Available information on nitrobenzene metabolism is based on animal experiments and fragmentary human data. There are two main metabolic pathways: (1) reduction to aniline followed hy hydroxylation to aminophenols and (2) direct hydroxylation of nitrobenzene to form nitrophenols. Further reduction of nitrophenols to aminophenols may also occur (Piotrowski, 1977). The rate of nitrobenzene metabolism is independent of the dose in later stages of acute or chronic intoxication. This can cause its accumulation in high-lipid tissues (Dorigan and Hushon, 1976).

The reduction of nitrobenzene to aniline occurs via the unstable intermediates, nitrosobenzene and phenyl hydroxylamine, both of which are toxic and have pronounced methemoglobinemic capacity. The reactions occur in the cytoplasmic and microsomal fractions of liver cells by the nitro-reductase enzyme system (Fouts and Brodie, 1957). This enzyme system is active in mice, guinea pigs, and rabbits, and is less active in rats and dogs. The aniline is then excreted as an acetyl derivative or hydroxylated and excreted as an aminophenol. Reddy, et al. (1976) showed that the gut flora of rats was needed for the reduction of nitrobenzene and subsequent methemoglobin formation.

The hydroxylation of nitrobenzene to nitrophenols does not occur in the microsomal fraction. The reaction proceeds via a peroxidase in the presence of oxygen (Piotrowski, 1977).

Robinson, et al. (1951) studied nitrobenzene metabolism in the rabbit using ^{14}C-labeled material. The main metabolic product found was p-amino-

phenol (35 percent) which was formed via phenylhydroxylamine. Seven phenols and aniline were detected as metabolites within 48 hours of a dose of 150 to 200 mg/kg body weight of nitrobenzene. Nitrobenzene was retained somewhat in the rabbits; its metabolites were detected in urine one week after dosing. Little unchanged nitrobenzene was excreted in the urine. The major urinary metabolites were p-aminophenol, nitrophenols, and nitrocatechol. These constituted 55 percent of the urinary metabolites and were excreted conjugated with sulfuric and glucuronic acids. About 1 percent of the dose was expired as radiolabeled carbon dioxide.

Yamada (1958) studied nitrobenzene metabolism in rabbits in a 3-month subcutaneous exposure study. He found that urinary excretion of detoxification products varied in the early stage of exposure, but did not in the later stages. The reduction and hydroxylation pathways all became depressed during the later stages of this chronic poisoning study.

Parke (1956) reports metabolites of nitrobenzene isolated four to five days after administering 0.25 mg/kg orally as a single dose in the rabbit (Table 2).

An investigation of the metabolism of ^{14}C-nitrobenzene in the cattle tick, Boophilus microplus, and spider, Nephia plumipes, was done by Holder and Wilcox (1973). They found that the tick metabolized nitrobenzene to nitrophenol and aniline whereas no free phenols were found as metabolites inthe spider. Aniline was the major metabolic product in both species.

Nitrobenzene, if present in sufficiently small amounts in water, can be degraded by some bacteria, such as Azobacter agilis. Nitrobenzene tends to inhibit its own degradation at concentrations above 0.02 to 0.03 mg/l (Dorigan and Hushon, 1976; Lu and Metcalf, 1975).

Lu and Metcalf (1975) studied nitrobenzene in a model aquatic ecosystem to assess biodegradation and biomagnification. The ecosystem consisted of

TABLE 2

Metabolic Fate of a Single Oral Dose (0.25 g/kg) of [^{14}C] Nitrobenzene in the Rabbit During 4-5 Days After Dosing[a]

Metabolite	Percentage of Dose (average)		
Respiratory CO_2	1		
Nitrobenzene	0.6*	2 in expired air	
Aniline	0.4+		
o-Nitrophenol	0.1		
m-Nitrophenol	9		
p-Nitrophenol	9		
o-Aminophenol	3		60 total
m-Aminophenol	4	58 in urine	
p-Aminophenol	31		
4-Nitrocatechol	0.7		
Nitroquinol	0.1		
p-Nitrophenyl Mercapturic acid	0.3		
(Total urinary radioactivity)	(58)		
Metabolized nitrobenzene in feces	9**		
Metabolized nitrobenzene in tissues	15-20		
Total accounted for	85-90%		

[a]Source: Parke, 1956
* 0.5% in the urine and 0.1% in the expired air.
+ 0.3% in the urine and 0.1% in the expired air.
** 6% of the dose was present in the feces as p-aminophenol.

green filamentous algae, Oedogonium cardiacium, snails, Physa, water fleas, Daphnia magna, mosquito larvae, Culex quinquifasciatus, and mosquito fish, Gambusia affinis, under controlled atmospheric conditions. ^{14}C-labeled nitrobenzene 0.005 to 0.5 mg/m^3 (0.01 to 0.1 ppm) was added to the water and animals were removed for analysis after 24 to 48 hours. The radiolabeled metabolites were extracted and separated by thin layer chromatography. The distribution of nitrobenzene and its degradation products is listed in Table 3.

Nitrobenzene was neither stored nor ecologicaly magnified, but was reduced to aniline in all organisms, acetylated in fish and water extracts only, and hydroxylated to nitrophenols by mosquito larvae and snails. The metabolites of nitrobenzene formed by the different organisms are illustrated in Figure 1.

Excretion

In man, the primary known excretion products of nitrobenzene are p-aminophenol and p-nitrophenol which appear in the urine after chronic or acute exposure. In experimental inhalation exposure to nitrobenzene, p-nitrophenol was formed with the efficiency of 6 to 21 percent. The efficiency of p-aminophenol formation is estimated from observation of acute poisoning cases where the molar ratio of excreted p-nitrophenol to p-aminophenol is two to one, since p-aminophenol is not formed at a detectable level in short subacute exposure (Piotrowski, 1977).

Ikeda and Kita (1964) measured the urinary excretion of p-nitrophenol and p-aminophenol in a patient admitted to a hospital with toxic symptoms resulting from a 17-month chronic industrial exposure to nitrobenzene. The results of their study are shown in Figure 2, which demonstrates that the rate of excretion of the two metabolites parallels the level of methemoglobin in blood. The authors exposed five adult rats to nitrobenzene vapor at

TABLE 3

Distribution of Nitrobenzene and Degradation Products in Model Aquatic Ecosystem*

		Nitrobenzene equivalents, ppm					
	Rf[a]	H_2O	Oedognoium (alga)	Daphnia (daphnia)	Culex (mosquito)	Physa (snail)	Gambusia (fish)
Total ^{14}C		0.53755	0.0690	0.1812	0.5860	0.6807	4.9541
Nitrobenzene	0.72	0.50681	0.0162	0.0709	0.3952	0.3886	4.0088
Aniline	0.60	0.01262	0.0032	0.0079	0.0272	0.0169	0.3527
Aminophenols[b]	0.20	0.00106	0.0080	0.0315	-	-	0.0986
Nitrophenols[b]	0.10	0.00466	0.0016	0.0394	0.1226	0.2190	0.0847
Polar	0.0	0.00896	0.0240	0.0315	0.0138	0.0393	0.1130
Unextractable		0.00164	-	-	-	-	-

*Source: Lu and Metcalf, 1975

[a] TLC with benzene:acetone:Skellysolve B (bp 60-68°C):diethylamine=65:25:25:5 (v/v).

[b] The isomers could not be separated reliably because of small amounts and similar R_f values.

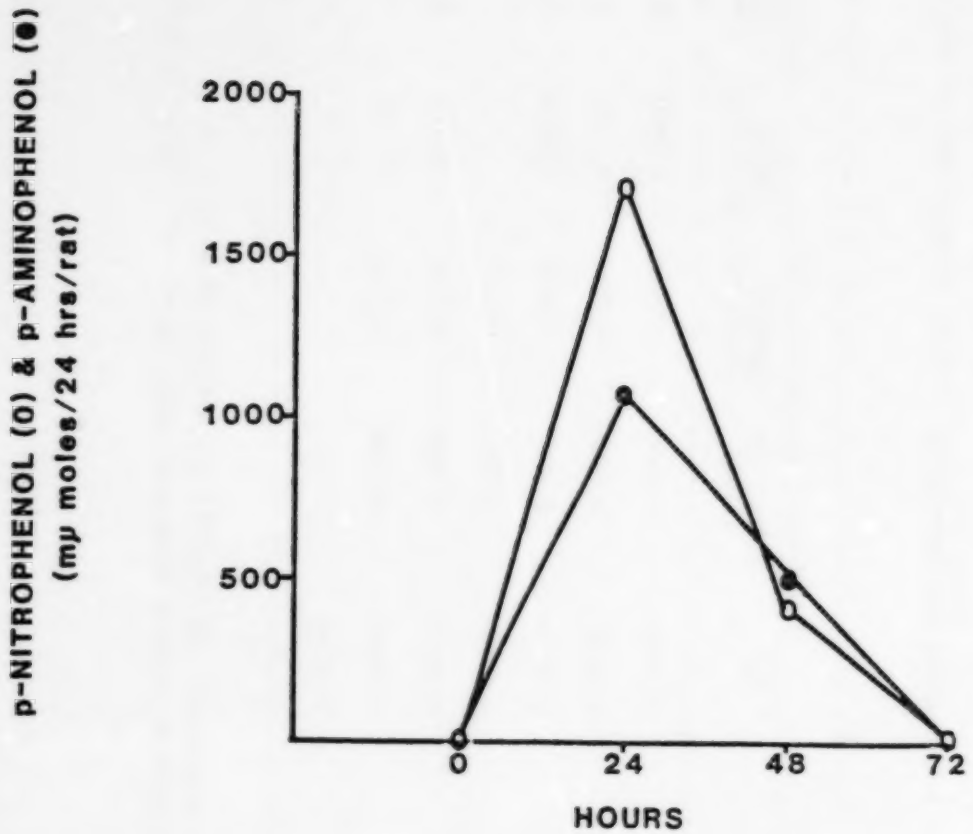


FIGURE 1

Relative detoxication capacities of key organisms of a model aquatic ecosystem following treatment with radioactive nitrobenzene.

Source: Lu and Metcalf, 1975

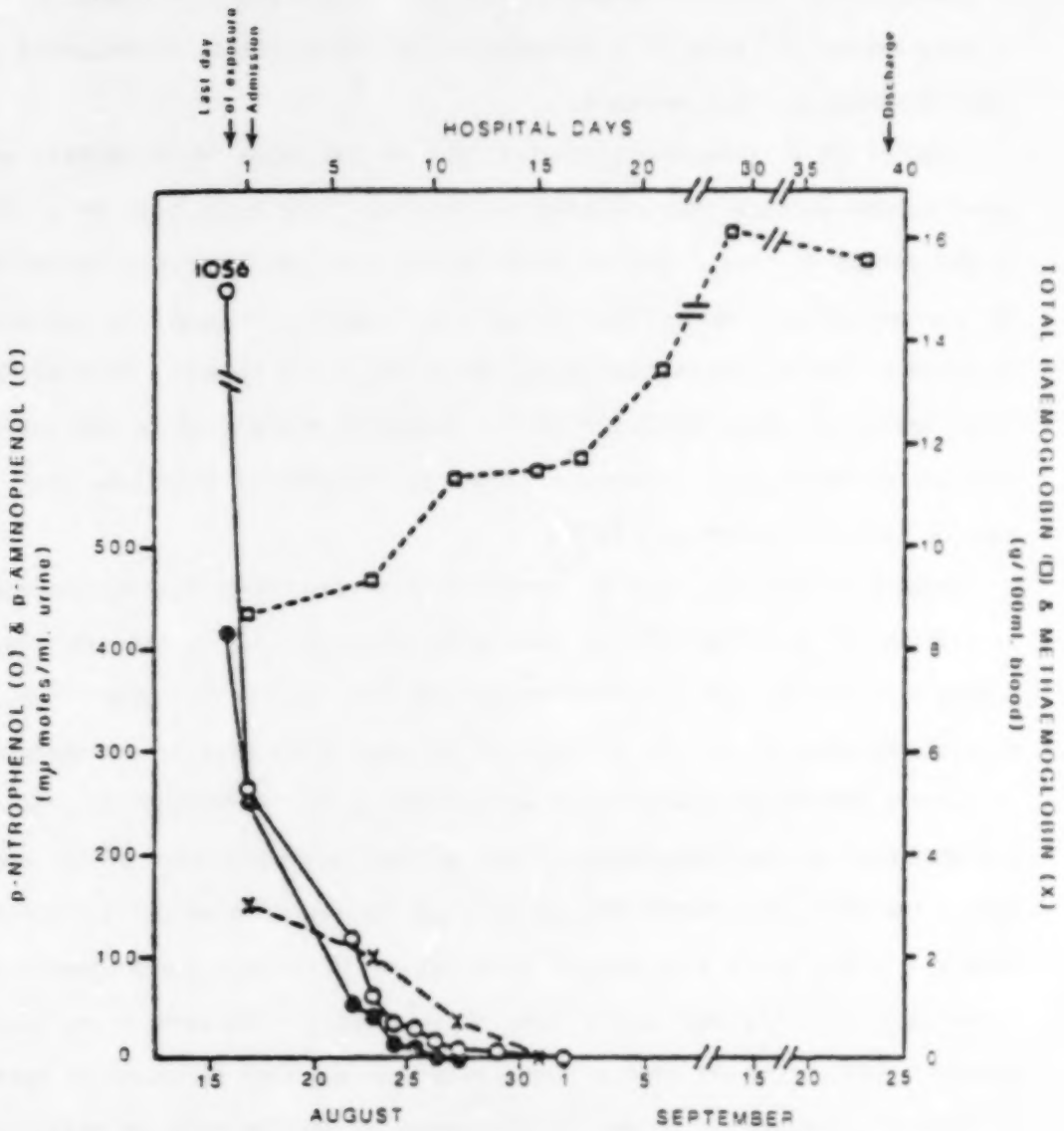


FIGURE 2

Changes in the levels of total hemoglobin and methemoglobin in blood and of p-nitrophenol and p-aminophenol in urine. The usual daily volume of urine was about 1 litre.

Source: Ikeda and Kita, 1964

125 mg/m^3 (25 ppm) for eight hours and measured the subsequent excretion of p-aminophenol and p-nitrophenol. The results are shown in Figure 3. The urinary excretion ratio of p-aminophenol and p-nitrophenol corresponded to their findings in the human case.

Studies of nitrobenzene concentrations in the blood of an acutely exposed person indicate that the compound remains in the human body for a prolonged period of time. Similar observations have been made from excretion of the two urinary metabolites in patients treated for acute or subacute poisoning. The excretion coefficient of urinary p-nitrophenol, followed for three weeks, is about 0.008 per hour. Metabolic transformation and excretion of nitrobenzene in humans is slower by an order of magnitude than in rats or rabbits (Piotrowski, 1977).

Because of the slow rate of nitrobenzene metabolism by humans, the concentration of p-nitrophenol in the urine increases for about four days during exposure and the concentration on the first day is only about 40 percent of the peak value. An estimate of the mean daily dose of nitrobenzene in chronic industrial exposure can be obtained by the measurement of urinary p-nitrophenol in specimens taken on each of the last three days of the work week. The level of nitrobenzene exposure can be approximated using the formula $y = 0.18z$, where y is the daily excretion of urinary p-nitrophenol in mg/day and z is the mean daily dose of absorbed nitrobenzene in mg (Piotrowski, 1967). The extended systemic retention and slow excretion of metabolites of nitrobenzene in man is determined by the low rate of metabolic transformation (reduction and hydroxylation) of the nitrobenzene itself. The conjugation and excretion of the metabolites, p-nitrophenol and p-aminophenol, is rapid (Piotrowski, 1977).

The urinary metabolites in man account for only 20 to 30 percent of the nitrobenzene dose; the fate of the rest of the metabolites is not known

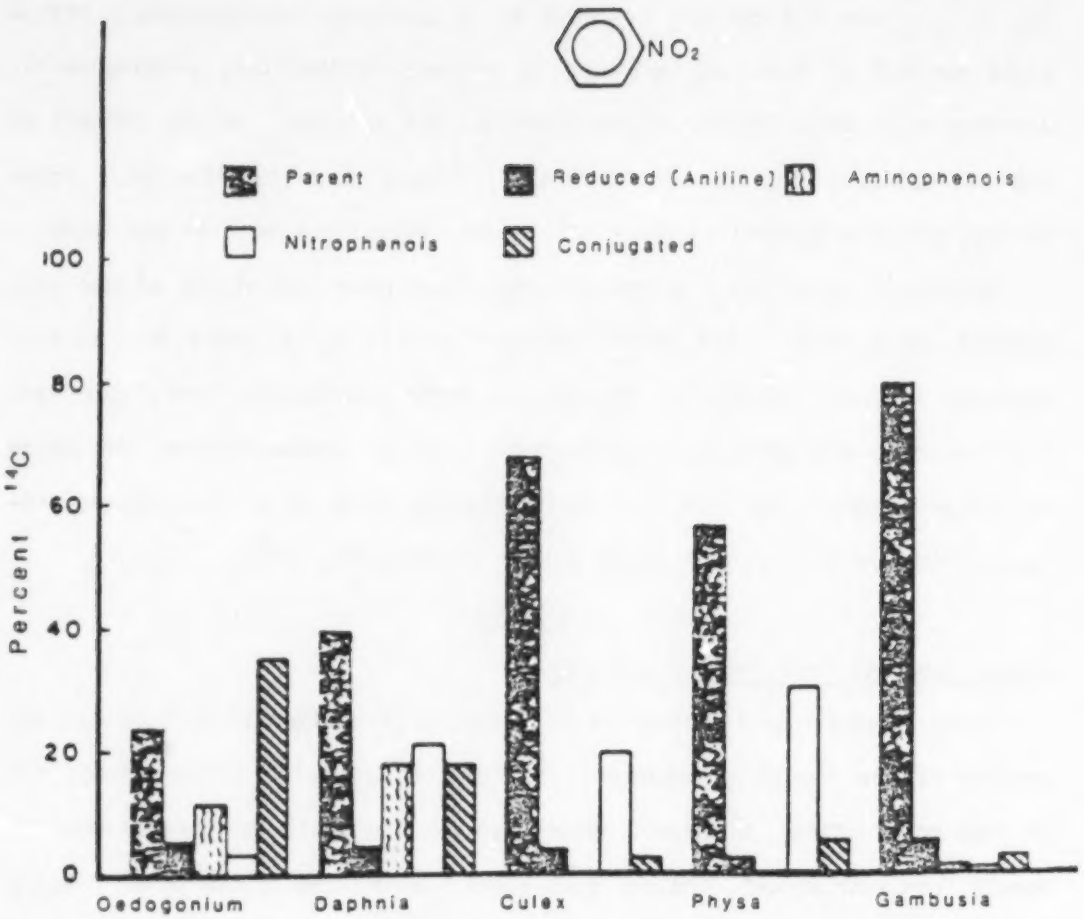


FIGURE 3

Excretion of p-nitrophenol and p-aminophenol in the urine of rats exposed to nitrobenzene.

Source: Ikeda and Kita, 1964

(Piotrowski, 1977). Parke (1956) studied ^{14}C-nitrobenzene metabolism in rabbits and was able to account for 85 to 90 percent of the dose which was administered by intubation. One percent of the nitrobenzene was exhaled as CO_2 in air, and 0.6 percent was exhaled as unchanged nitrobenzene. Fifty-eight percent of the dose appeared as urinary metabolites, p-aminophenol, nitrophenols, aminophenols, nitrocatechols, and aniline. Thirty percent of the nitrobenzene remained in the rabbit tissue four to five days after dosing, and nine percent of the nitrobenzene metabolites were in the feces.

Urinary p-nitrophenol in man is determined after hydrolysis of the conjugated metabolites. Analytical methodology (of which there are several methods) involves removal of interfering color substances, hydrolysis, extraction of p-nitrophenol, re-extraction into an aqueous system, reduction to a p-aminophenol, and reaction to indophenol, which is a blue colored product. The sensitivity is 5 ug per sample (Piotrowski, 1977).

EFFECTS

Acute, Subacute, and Chronic Toxicity

Acute exposure to nitrobenzene can occur from accidental or suicidal ingestion of the liquid nitrobenzene or ingestion as false bitter almond oil in food or medicine. Cutaneous absorption causing acute toxic reactions can result from wearing wet, freshly dyed shoes (Levin, 1927); use of on diapers or protective pads (Etteldorf, 1951); use of soap or skin oil containing nitrobenzene (Zeitoun, 1959); or from an untreated spill of nitrobenzene on the skin in an industrial plant (Hamilton, 1919). The fatal dose of nitrobenzene in humans varies widely; values from less than 1 ml to over 400 ml have been reported (Wirtschafter and Wolpaw, 1944). Chronic toxic effects in man generally result from industrial exposure to vapor that is absorbed

through the lungs or the skin. One case of chronic toxicity was reported in a woman who used nitrobenzene as a cleaning solution for many years (Hamilton, 1919).

Symptoms of chronic occupational nitrobenzene absorption are cyanosis, methemoglobinemia, jaundice, anemia, sulfhemoglobinemia, presence of Heinz bodies in the erythrocytes, dark colored urine, and the presence of nitrobenzene metabolites (e.g., nitrophenol) in the urine (Pacseri and Magos, 1958; Hamilton, 1919; Wuertz, et al. 1964; Browning, 1950; Malden, 1907; Piotrowski, 1967).

The symptoms of dinitrobenzene poisoning include those found in nitrobenzene toxicity as well as abdominal pain, weakness, enlarged liver, and basophilic granulations of red corpuscles (Beritic, 1956; Malden, 1907). Dinitrobenzene poisoning also causes unequal responses in different exposed workers.

The outstanding symptom of acute nitrobenzene poisoning is cyanosis as a result of methemoglobin formation (up to 80 percent) (Piotrowski, 1967). If the cyanosis is severe or prolonged the patient will go into coma and may die. Often anemia is seen a week or two after acute poisoning as a result of the hemolytic effect of nitrobenzene (Stevenson and Forbes, 1942). Suicidal ingestion of nitrobenzene has been reported (Nabarro, 1948; Leinoff, 1936; Myslak, et al. 1971), and the compound has also been used unsuccessfully to induce abortion (Nabarro, 1948; Dorigan and Hushon, 1976). Harrison (1977) reported a case of poisoning from an aniline-nitrobenzene mixture which was accidentally ingested from a pipette by a chemistry student. The mortality due to ingested nitrobenzene in the above cases was variable, depending on the health of the patients and the treatments they received. Common treatments include gavage, transfusions, oxygen therapy, methylene blue, ascorbic acid, and toluidine blue. Treatment is usually directed to

reduce the methemoglobinemia which is the immediate effect, and often the cause of death, in nitrobenzene poisoning. Death has resulted from intake of less than one ml of nitrobenzene (Wirtschafter and Wolpaw, 1944).

Some of the reported toxicity values are summarized in Table 4 (Fairchild, 1977). The term LD_{Lo} designates the lowest reported lethal dose and TD_{Lo} is the lowest published toxic dose.

Levin (1927) demonstrated *in vivo* production of methemoglobin by nitrobenzene in dogs, cats, and rats, but not in guinea pigs or rabbits. Dresbach and Chandler (1918) found that nitrobenzene fumes caused cerebellar disturbances in dogs and birds, while blood changes were the principal toxic effects in other mammals they studied. Reddy, et al. (1976) reported a delay in methemoglobin formation in germ free rats by nitrobenzene and postulated that the gut flora of rats was responsible for the reduction of (*in vivo*) and methemoglobin forming capacity of nitrobenzene. Shimkin (1939) measured the toxicity of nitrobenzene in mice when absorbed through the skin. He found the minimum lethal dose to be 0.0004 ml/gm body weight by a subcutaneous route of administration. The nitrobenzene caused respiratory failure, reduction of the white blood cell count, and liver pathology in the mice.

Yamada (1958) did a chronic toxicity study in rabbits that received a subcutaneous dose of 840 mg/kg body weight per day for three months. He found a decrease in erythrocyte number and hemoglobin content early in the exposure. These values increased during the three months but did not return to normal levels. Urinary excretion of detoxification products was variable in the early stages of the exposure, but then all the detoxification reactions (reduction, hydroxylation, and acetylation) were depressed. As a result of these observations, Yamada divided this response in the rabbit into three stages: initial response, resistance, and exhaustion.

The effects of subacute nitrobenzene exposure in rats were studied by Kulinskaya (1974). Vasilenko and Zvezdai (1972) measured blood changes and found sulfhemoglobin formation to be the most regular and persistant change noted. Increased methemoglobin levels with Heinz body formation and anemia were also seen.

The cytotoxicity of nitrobenzene to cultured Erlich-Landschutz diploid (ELD) cells was measured by Holmberg and Malmfors (1974). They found no significant increase in cell injury after five hours incubation with nitrobenzene. However, a 3M nitrobenzene solution reduced cell proliferation by 50 percent in cultured hamster cells (Raleigh, et al. 1973). Oxygen consumption by cultured cells is increased by nitrobenzene (Biaglow and Jacobson, 1977). Its derivatives are used to sensitize malignant cells in vitro to radiation effects (Chapman, et al. 1974). The authors suggest that this effect was due to radical oxidation and increased cellular damage.

Nitrobenzene derivatives have a wide variety of toxic effects. 1-Chloro-2,4-dinitrobenzene (DNCB) is a well known skin sensitizer in guinea pigs, mice, and humans (Hamaguchi, et al. 1972; Jansen and Bleumink, 1970; Maurer, et al. 1975; Weigand and Gaylor, 1974; Noonan and Halliday, 1978). Cooke, et al. (1968) showed that nitrobenzene derivatives react with cell membranes to alter sodium-potassium conductance, and sometimes affect action potentials of nerve cells.

m-Dinitrobenzene is a potent methemoglobin former, and is more toxic than nitrobenzene (Ishihara, et al. 1976; Pankow, et al. 1975). Pentachloronitrobenzene (PCNB) is a common fungicide with varying toxic effects in different mammalian species (Courtney, et al. 1976).

Some of the toxic effects of nitrobenzene are summarized in Appendix A (Dorigan and Hushon, 1976).

TABLE 4

Acute Toxicity Values*

Animal	Route	Toxic Dose	
Human (female)	oral	TD_{Lo}:	200 mg/kg
Human	oral	LD_{Lo}:	5 mg/kg
Rat	oral	LD_{50}:	640 mg/kg
Rat	skin	LD_{50}:	2,100 mg/kg
Rat	i.p.	LD_{50}:	640 mg/kg
Rat	s.c.	LD_{Lo}:	800 mg/kg
Mouse	s.c.	LD_{Lo}:	286 mg/kg
Dog	oral	LD_{Lo}:	750 mg/kg
Dog	i.v.	LD_{Lo}:	150 mg/kg
Cat	oral	LD_{Lo}:	2,000 mg/kg
Cat	skin	LD_{Lo}:	25 g/kg
Rabbit	oral	LD_{Lo}:	700 mg/kg
Rabbit	skin	LD_{Lo}:	600 mg/kg
Guinea pig	i.p.	LD_{Lo}:	500 mg/kg

*Source: Fairchild, 1977

Aquatic toxicity: TL_m at 96 hours: 10-100 mg/l (ppm).

Synergism and/or Antagonism

Alcohol has a synergistic effect on nitrobenzene poisoning. Ingestion of an alcoholic beverage induced immediate acute toxic symptoms, including coma, in a worker who had apparently recovered from the effects of chronic nitrobenzene exposure. Alcohol ingestion or chronic alcoholism can lower the lethal or toxic dose of nitrobenzene (Dorigan and Hushon, 1976). In subchronic dinitrobenzene poisoning, drinking of one beer or exposure to sun can bring on an acute crisis as late as six weeks after the disappearance of other symptoms (Rejsek, 1947).

Smyth, et al. (1969) studied the synergistic action between nitrobenzene and 27 other industrial chemicals by intubation in rats. Most of the compounds tested did not alter the LD_{50}. In another study, ingestion of 2 to 20 ml of ethanol increased the severity of reaction to a 0.1 ml intravenous dose of nitrobenzene in rabbits. This observation agrees with the clinical data on the synergism of ethanol and nitrobenzene (Dorigan and Hushon, 1976).

Kaplan, et al. (1974) studied the effect of caffeine, an inducer of microsomal enzymes, on methemoglobin formation by nitrobenzene in rats. Methemoglobin was formed and then decreased in induced animals. The increased microsomal enzyme level increased the rate of metabolism and excretion of nitrobenzene and thus caused a rapid decline of methemoglobin levels.

Teratogenicity

There is a paucity of information on the teratogenic effects of nitrobenzene. In one study (Kazanina, 1968b), 125 mg/kg was administered subcutaneously to pregnant rats during preimplantation and placentation periods. Delay of embryogenesis, alteration of normal placentation, and abnormalities in the fetuses were observed. Gross morphogenic defects were seen in four of 30 fetuses examined.

Changes in the tissues of the chorion and placenta of pregnant women who used nitrobenzene in the production of a rubber catalyst were observed. No mention was made of the effects on fetal development or viability (Dorigan and Hushon, 1976). Menstrual disturbances after chronic nitrobenzene exposure have also been reported.

Garg, et al. (1976) tested substituted nitrobenzene derivatives for their ability to inhibit pregnancy in albino rats. Two of the compounds tested (p-methoxy and p-ethoxy derivatives) inhibited implantation 100 percent when administered on days one through seven after impregnation.

The available data, although sketchy, indicate that women who are or wish to become pregnant should avoid exposure to nitrobenzene. Further studies of nitrobenzene teratogenicity in mammals are needed.

Mutagenicity

Chiu, et al. (1978) tested nitrobenzene and 53 commercially available heterocyclic and aliphatic nitro- compounds for mutagenicity using the Ames Salmonella typhimurium strains TA 98 and TA 100. They reported that 34 of the 53 compounds tested were mutagenic. Nitrobenzene was not found to be mutagenic.

Trinitrobenzene was mutagenic in two in vitro assays, the Ames Salmonella microsomal assay and the mitotic recombination assay in yeast (Simmon, et al. 1977). Other nitrobenzene derivatives have demonstrated mutagenicity in in vitro assays, so that the mutagenicity of nitrobenzene is still in question and additional work is needed in this area.

Carcinogenicity

The available literature does not demonstrate the carcinogenicity of nitrobenzene, however, some nitrobenzene derivatives have demonstrated carcinogenic capacities. For example, pentachloronitrobenzene (PCNB) has induced hepatomas and papillomas in mice (Courtney, et al. 1976).

1-Fluoro-2,4-dinitrobenzene (DNFB) was demonstrated by Bock, et al. (1969) to be a promoter of skin tumors in mice, although it does not induce them when administered alone.

Carcinogenic activity is frequently a general characteristic of structurally related compounds (Arcos and Argus, 1974). Because of the structural similarity of nitrobenzene to the above nitrobenzene derivatives, nitrobenzene should be regarded as a suspect carcinogen. The same conclusion, based on more circumspect reasoning, was reached by Dorigan and Hushon (1976). This suspicion, while strong enough to warrant the testing of nitrobenzene for carcinogenicity, is not sufficiently strong to recommend a criterion based on carcinogenicity.

CRITERION FORMULATION

Existing Guidelines and Standards

The maximum allowable concentration of nitrobenzene in air in industrial plants is 5 mg/m^3. This value was set by the joint ILO/WHO Committee on Occupational Health in 1975 (Goldstein, 1975). The OSHA standard for nitrobenzene in air is 5 mg/m^3 (1 ppm) set in 1977 (40 CFR 1910.1000). This is also the limit in Germany and Sweden while the exposure limit in the USSR is 3 mg/m^3 (Dorigan and Hushon, 1976).

There are no standards for nitrobenzene levels in water. Nitrobenzene was not listed among the substances for which a maximum water concentration has been set.

Current Levels of Exposure

Extrapolating from Piotrowski's exposure data, a worker exposed to the current occupational standard of 5 mg/m^3 (1 ppm) nitrobenzene for an eight-hour work day would absorb approximately 24 mg by inhalation and 9 mg cutaneously. The maximum eight-hour uptake would be 33 mg, which is less than the "reasonable safe" level of 35 mg/day (Dorigan and Hushon, 1976). Doses of up to 70 mg/day have been reported for factory workers and up to 80 mg/day have been reported in a dye stuff factory in England (Piotrowski, 1967).

Nitrobenzene can be a contaminant in industrial wastewater, and companies utilizing or producing nitrobenzene are required to monitor its level in their effluent waste. Using gas chromatography the minimum detectable level of nitrobenzene in drinking water is 0.7 ng (Austern, et al. 1975).

Nitrobenzene may be vented to the atmosphere. The vents are usually equipped with absorbers or scrubbers, but some nitrobenzene vapor can escape. Atmospheric nitrobenzene levels outside a plant are not monitored

by industry. Since inner plant levels are below the standard of 5 mg/m^3 (1 ppm) and nitrobenzene vapor accumulates at the floor level due to its high density, the external air nitrobenzene concentrations are expected to be very low (Dorigan and Hushon, 1976).

Special Groups at Risk

Workers in plants producing or using nitrobenzene have the greatest risk of toxic exposure. At the current OSHA standard of 5 mg/m^3 (1 ppm), a worker could absorb as much as 33 mg/day. This is enough to produce symptoms of chronic toxicity in some susceptible individuals (Dorigan and Hushon, 1976). The amount of nitrobenzene absorbed by a worker via inhalation and cutaneous absorption can be estimated from the level of total (free and conjugated) p-nitrophenol in urine as described by Piotrowski (1977).

Due to the current widespread use of disposable diapers and underpads in hospitals, nitrobenzene poisoning in infants from laundry marking dyes, in most cases, has been studied and corrected.

Pregnant women may be especially at risk with respect to nitrobenzene due to transplacental passage of the agent. Individuals with glucose-6-phosphate dehydrogenase deficiency may also be at special risk (Calabrese, et al. 1977; Djerassi, et al. 1975). Additionally, because alcohol ingestion or chronic alcoholism can lower the lethal or toxic dose of nitrobenzene (Rejsek, 1947; von Oettingen, 1941), individuals consuming alcoholic beverages may be at increased risk.

Basis and Derivation of Criterion

There are no established standards for nitrobenzene in water. Because there are little or no data available on the toxicity of nitrobenzene ingested in drinking water, or on the teratogenic, mutagenic, or carcinogenic effects of nitrobenzene in general, experimental testing is necessary before

a criterion can be derived from oral ingestion data. It is recommended that testing in these areas of toxicity be implemented so that the effects of nitrobenzene on mammals may be better understood.

Until more toxicological data on oral ingestion in animals are generated, criterion levels must be estimated from occupational exposure data and from organoleptic data. As reported, nitrobenzene produces a detectable odor in water at a threshold (lowest discernible concentration) of 30 µg/l (Austern, et al. 1975; U.S. EPA, 1970; Alekseeva, 1964). It should be noted, however, that this criterion level is based on aesthetics rather than health effects.

A water quality criterion (WQC) can be derived from the Threshold Limit Value (TLV) of 5 mg/m^3. This can be done by estimating the total daily dose allowed by the TLV from both inhalation and dermal exposure. An inhalation absorption coefficient of 0.8 will be used based on data provided by Piotrowski (1967, 1977). Assuming an air intake of 10 m^3/work day, the portion of allowable dose by inhalation is 40 mg (5 mg/m^3 x 10 m^3/work day x 0.8). The portion of the allowable dose by dermal exposure can be calculated from the 7:18 ratio of dermal:inhalation exposure estimated by Piotrowski (1967, 1977), i.e., 7/18 x 40 mg/work day = 16 mg/work day. Thus the total allowable dose per work day is 56 mg (40 mg + 16 mg). The allowable daily intake (ADI) can be calculated by adjusting for a 5/7 day work week, i.e., 56 mg/work day x 5/7 = 40 mg/day.

Assuming 100 percent gastrointestinal absorption of nitrobenzene, a daily water consumption of 2 liters, a daily fish consumption of 0.0065 kg, and a bioconcentration factor of 2.89, the water quality criterion is:

$$\frac{40 \text{ mg}}{2 \text{ liters} + (2.89 \times 0.0065)} = 19.8 \text{ mg/l}$$

Since the WQC using the TLV is well above the detectable odor level of nitrobenzene, water containing this concentration of nitrobenzene would not be aesthetically acceptable for drinking. Even though the limitations of using organoleptic data as a basis for establishing a WQC are recognized, it is recommended that a WQC of 30 µg/l be established at the present time. This level may be altered as more data are developed upon which to calculate a WQC.

The analysis and recommendations generated in this document are based on the literature available to date. If future reports indicate that nitrobenzene may be carcinogenic, mutagenic or teratogenic, a reassessment of the WQC will be necessary.

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APPENDIX

Toxicological Effects of Nitrobenzene

Organism	Route	Exposure	Exposure Time	Response
Human	Inhalation	-	8 hrs./day for 17 mos. factory worker	Cyanosis, headache, fatigue methemoglobinemia (Ikeda and Kita, 1964).
	Inhalation	Poor ventilation	8 hrs./day for 1.5 mos. factory worker paint firm	Cyanosis, headache, fatigue, methemoglobinemia, liver damage, hypotension (Ikeda and Kita, 1964).
			8 hrs./day for 4.5 mos.	Above plus: liver and spleen enlarged and tender, hyperalgesia in extremeties (Ikeda and Kita, 1964).
	Inhalation	0.2-0.5 mg/l (40-100 ppm)	ca. 6 hours.	Slight effects, e.g., headache, fatigue (von Oettingen, 1941).
	Inhalation	0.129 mg/m^3	-	Threshold level for electroencephalograph disturbance (Andreeshcheva, 1964).
	Inhalation	"Large" amounts poor ventilation		Hospitalized: Day 1 - fatigue, headache, asthma 2 - vertigo, coma, cyanosis 3 - labored breathing, urine with almond odor 7 - methemoglobinemia recovery after 1 mo. (Ravault, et al. 1946).
	Inhalation	Acute	-	Burning throat, nausea, vomiting, gastrointestinal disturbances, cold skin, livid face, cyanosis (von Oettingen, 1941).

APPENDIX (Continued)

Organism	Route	Exposure	Exposure Time	Response
Human	Inhalation	-	- Nitrobenzene factory worker	Intermittent symptoms: cyanosis, pallor and jaundice, pharyngeal congestion, headache, changes in blood cell composition (increased polynuclears and eosinophils (von Oettingen, 1941).
	Inhalation	6-30 µg/l	6 hrs.	Retained 80% of vapor in lungs, urinary excretion of p-nitrophenol (maximum in 2 hrs., still detected after 100 hrs.) (Salmowa, et al. 1963).
	Inhalation	-	- Factory worker (rubber accelerator)	Pregnant women: thickening of tissue in blood vessels, decreased placental absorption, necrosis in placental tissue (Ferster, 1970).
	Inhalation	-	Factory worker (glass, porcelain)	Changes in bone marrow, increased lymphoid cell production, impairment of copper metabolism and certain iron-containing enzymes (Yordanova, et al. 1971).
	Inhalation	-	- Industrial exposure	Disturbance of motor impulses (Zenk, 1970).
	Inhalation	Acute	- Factory worker (filled containers with nitrobenzene)	14 days: cyanosis, headache, backache, stomach ache, vomiting ca. 21 days: drank beer and fell unconscious, cyanosis, dilated pupils, retarded respiration, weak pulse 1 yr.: intelligence dimmed 2 yrs.: emaciated, atrophied muscles

APPENDIX (Continued)

Organism	Route	Exposure	Exposure Time	Response
Human				3 yrs.: memory failed 6 yrs: loss of perception of time and space (Korsakoff's syndrome) (Chandler, 1919).
	Cutaneous absorption	Dye used in diaper stamps	-	Babies: cyanosis, rapid pulse, shallow respiration, vomiting, convulsions, recovery in 24 hrs. (von Oettingen, 1941).
	Cutaneous absorption	Shoe dye	ca. 7 hrs.	Unconsciousness after consumption of alcoholic beverages, death (Chandler, 1919).
	Cutaneous absorption	0.5% by weight in paper	- (Handled carbon paper	Dermatitis (Calan and Connor, 1972)
	Oral	-	From human milk	Nurselings became cyanotic, recovery in 24 hrs. (mothers ate almond cake artificially flavored with nitrobenzene) (Dollinger, 1949).
	Oral	333 ml	Single	Maximum dose with recovery reported following severe symptoms (von Oettingen, 1941).
	Oral	0.4 ml	Single	Minimum lethal dose reported (von Oettingen, 1941).
Rabbit	Subcutaneous injection	0.8 mg/kg	Daily for 3 mo.	Maximum dose not causing death (Yamada, 1958).

APPENDIX (Continued)

Organism	Route	Exposure	Exposure Time	Response
Rabbit	Subcutaneous injection	10-14 mg/kg	Single	Minimum dose producing observable effects; slow and lasting methemoglobinemia (von Oettingen, 1941)
	Cutaneous absorption	700 mg/kg	Single	After 52 hrs.: lethal (von Oettingen, 1941)
	Intraperitoneal injection	500 mg/kg	Single	Reduced blood pressure and myocardial glycogen level (Labunski, 1972).
	Intravenous	100 mg	Daily or every 5 days	Simultaneous doses of 2-20 ml ethanol increased severity of poisoning (Matsumara and Yoshida, 1959).
	Oral	9 gm	4 doses, one every 15 minutes	Convulsions, death (von Oettingen, 1941; Chandler, 1919).
	Oral	4.8 gm	Single	Lethal instantly (von Oettingen, 1941; Chandler, 1919).
	Oral	700 mg/kg	Single	Lethal dose (Stecher, 1968).
	Oral	600 mg	Single	Dizziness, loss of reflexes, methemoglobinemia, congestion of brain tissue - 12 hrs. - death (Chandler, 1919).
	Oral	300 mg	Single	Fatigue for 1 week (Parke, 1956).
	Oral	50 mg/kg	Single	Tissue degeneration, especially heart, liver, kidney (Papageorgiou and Argoudelis, 1973)

APPENDIX (Continued)

Organism	Route	Exposure	Exposure Time	Response
Rabbit	Oral	1 mg/kg	Single	Lowered hemoglobin, erthyrocytes and lymphocytes; increased leucocytes (Kazakova, 1956).
	Oral	0.1 mg/kg	Single	Threshold toxic dose (Kazakova, 1956)
Guinea pig	Inhalation	Saturated air (0.04 vol. %)	2-5 hrs.	Death following tremors, paralysis of hind legs (Chandler, 1919).
	Subcutaneous	0.2 gm/kg	Every other day for 6 mos.	Hemolytic anemia, loss of weight, decreased motor activity, fluxes in urinary excretion of 17-hydroxy-corticosteroids (Porter-Silber chromogens) (Makotchenko and Akhmetov, 1972).
	Oral	ca. 3 gm	Single	0.5 hrs: tremors, faint heartbeats, labored respiration 2 hrs: death (Chandler, 1919).
	Oral	ca. 1.2 gm	Single	Immediately motionless, then complete recovery (Chandler, 1919).
	Oral	50 mg/kg	1 year	Tissue degeneration, especially heart, liver, kidney (Kazakova, 1956).
	Oral	1 mg/kg	Single	Lowered hemoglobin, erythrocytes, lymphocytes; increased leucocytes (Kazakova, 1956).
	Oral	0.1 mg/kg	Single	Threshold toxic dose (Kazakova, 1956).

APPENDIX (Continued)

Organism	Route	Exposure	Exposure Time	Response
Rat	Inhalation	5 mg/m^3	8 hrs.	Metabolites excreted in 3 days (Ikeda and Kita, 1964).
	Inhalation	ca. 0.03 mg/m^3	Daily, up to 98 days	Increased ability to form sulfhemoglobin in preference to methemoglobin (Andreeshcheva, 1970).
	Inhalation	0.06-0.1 mg/m^3	70-82 days	Cerebellar disturbances, inflamed internal organs (Khanin, 1969).
	Inhalation	0.008 mg/m^3	73 days	No effect (Andreeshcheva, 1964).
	Oral	600 mg/kg	Single	LD_{50} (Smyth, et al. 1969).
	Intraperitoneal injection	800 mg/kg	Single	Lethal (Magos and Sziza, 1958).
	Subcutaneous injection	640 mg/kg	Single	Blood catalase activity decreased continuously over 96 hrs. (Goldstein and Popovici, 1959).
	Subcutaneous injection	300 mg/kg	Single	LD (14 days) - methemoglobinemia, anemia, sulfhemoglobinemia (Brown, et al. 1975).
	Subcutaneous injection	200 mg/kg or 100 mg/kg	Single Daily for 10 days	Methemoglobinemia, sulfhemoglobinemia, anemia (Zvezdai, 1972).
	Subcutaneous injection	125 mg/kg	Single	Delayed embryogenesis, abnormal fetal development and embryo death changes in polysaccharide composition of placenta (Kazanina, 1967, 1968a,c).

APPENDIX (Continued)

Organism	Route	Exposure	Exposure Time	Response
Rat	Subcutaneous injection	100-200 mg/kg	Single	Sulfhemoglobin (most regular and persistent form of hemoglobin) nitroxyhemoglobin, increased methemoglobin (Vasilenko and Zvezdai, 1972).
Mouse	Cutaneous absorption	480 mg/kg	-	30 min: prostrate, motionless 24 hrs: death (von Oettingen, 1941)
	Intraperitoneal injection	1.23 gm/kg	Single	40 min.: 67% dead (Smith, et al. 1967).
	Intraperitoneal	1 gm/kg	Single	10-15 min: incoordination, comatose shallow respiration Several hrs.: regained coordination Immediately before death: lost coordination again, respiratory arrest 48 hrs: death (Smith, et al. 1967).
	Intraperitoneal injection	20 mg/kg	Single	Lethal dose (Brown, et al. 1975).
	Intraperitoneal injection	12.3 mg/kg	Single	10 min.: 4.2% methemoglobin formed (Smith, et al. 1967).
Cat	Inhalation	Saturated air (0.04 vol. %)	2-5 hrs.	Death following tremors, paralysis of hind legs (Chandler, 1919).
	Inhalation	-	2-3 hrs.	Death
	Oral	2.4 gm	Single	Death in 12-24 hrs. (von Oettingen, 1941; Chandler, 1919).

APPENDIX (Continued)

Organism	Route	Exposure	Exposure Time	Response
Dog	Inhalation	"Thick vapor"	1.5 hrs.	Complete anesthesia and sleep (Chandler, 1919).
	Intravenous injection	150-250 mg/kg	Single	Minimum lethal dose - lowered blood pressure, pulse rate increased then decreased; respiration stimulated until paralyzed (von Oettingen, 1941).
	Oral	28.8 gm plus 6 gm	2 doses, 0.5 hrs. apart	Immediate: agitation, then motionless 1 hr.: convulsions, then motionless 4.5 hrs.: tremors, hind legs paralyzed 18 hrs.: death (Chandler, 1919).
	Oral	24 gm	Single	Few hrs.: "stupid" 12 hrs.: deep coma, slow respiration, lowered skin temperature, stomach strongly alkaline (Chandler, 1919).
	Oral	2.4 gm	Single	1 hr: vomiting, then sleep continuing for 6 hrs. 6 hrs: appeared normal 15-68 hrs: rigid muscles 104 hrs: death (Chandler, 1919).
	Oral	750-1000 mg/kg	Single	Minimum lethal dose (von Oettingen, 1941).
	Oral	500-700 mg/kg	Single	Salivation, unrest, dizziness, tremors, increased pulse rate, sometimes convulsions (Chandler, 1919)

APPENDIX (Continued)

Organism	Route	Exposure	Exposure Time	Response
Dog	Oral	-	Daily	Formed methemoglobin continuously at "certain" concentration (Hashimoto, 1958).
Chicken	Oral	1.2 gm	Single	Unsteady gait, recovery (Chandler, 1919).
	Oral	2.4 gm	Single	Immediately unconscious 12 hrs.: death (Chandler, 1919).
Pigeon	Inhalation	-	1 hr. 2-3 hrs.	No effects Death (Chandler, 1919).